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Morphometric Analysis of Four Species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) Attacking Codling Moth and other Tortricid Pests in North America

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Abstract.—Four species of *Trichogramma* Westwood are distinguished with overlap using morphometric analyses of 496 specimens with 27 measurements of males, 26 of females. The cryptic species of the *T. minutum* complex, *T. minutum* Riley and *T. platneri* Nagarkatti, are distinguished morphologically for the first time using canonical variate analysis. Intermediacy between *T. californicum* Nagaraja and Nagarkatti and the *T. minutum* complex is examined, with reference to other sources of variation. Males of the four species could be identified using a linear discriminant function with 0–3.9% error when applied to specimens used in developing the function, with *T. minutum* and *T. californicum* identified with error rates of 10.3–12.8% using novel resampled data; females could be separated with error rates of 3.3–18.9% using only the calibration specimens, with *T. minutum* and *T. californicum* identified with an error rate of 27.2–29.4% using novel resampled data. An identification key to males of the four species is provided that uses a combination of morphological characters and the discriminant functions. Implications of these results for quality control of mass releases and the identification of *Trichogramma* are discussed.

Trichogramma Westwood is the most important genus of egg parasitoids attacking Tortricidae in tree crops (Mills and Carl 1991). They are routinely released in augmentative biological control programs, although with mixed success (Falcon and Huber 1991, Smith 1996). By far the most common wasps used in the augmentative control of these pests in North America are the two species that comprise the *T. minutum* species complex: *T. minutum* Riley and *T. platneri* Nagarkatti. These species are also the dominant native egg parasitoids of tortricid pests in fruit orchards (Stouthamer et al. 2000b). At least nine other native species of *Trichogramma* attack these pests, but these appear to be much less common (Pinto et al. 2002).

The *T. minutum* species complex presents one of the most acute taxonomic problems in the genus, because its two species have been considered indistinguishable morphologically (Nagarkatti 1975, Pinto et al. 1991). Their separation is based on mutual reproductive incompatibility, differences in certain allozymic loci as determined through electrophoresis (Pinto et al. 1992) and geography, with *T. minutum* found primarily east of the Rocky Mountains and *T. platneri* west of the Rockies. The two species mate readily in laboratory settings, but hybrid females die in the embryonic stage (Stouthamer et al. 2000b). Because both species are commonly mass released against Lepidopteran pests (Kuhlmann and Mills 1999), these findings have serious implications for augmentative control programs, as it is clear that unless high rates of sib-mating or intraspecific encounters between individuals of different sexes occur, there can be negative reproductive interactions when releases result in mixed populations of the two species. Sib-mating should be particularly low for parasitoids of small, isolated eggs, and it has been estimated

that levels of sib-mating for the *T. minutum* complex on codling moth, *Cydia pomonella* (Linnaeus), and oriental fruit moth, *Grapholita molesta* (Busck), on tree crops will not exceed 63%, resulting in considerable opportunity for interspecific mating when both species are present (Stouthamer et al. 2000b). For these reasons, the accurate identification of insectary cultures and field populations is imperative to maximize the benefit of control programs involving members of the *T. minutum* complex.

Morphological identification of most species of *Trichogramma* is difficult due to their small size and overlap of potentially diagnostic characters (Pinto 1999). Almost all of the diagnostic characters for *Trichogramma* are found on either the genitalia or antennal flagellum of males. Female *Trichogramma* have been considered unidentifiable morphologically except when the number of possible diagnoses can be narrowed down by associating them with co-occurring males of known identity. This is a major problem considering that females are usually more common than males, and males are absent in thelytokous populations. Non-morphological identification methods are available, including reproductive compatibility with known reference cultures and diagnostic allozymic profiles (Pinto et al. 1991, 1992), but these methods require living and specially preserved dead specimens, respectively, and usually the establishment of cultures to provide enough material for study. The ITS2 genetic region has been investigated as a means of separating *T. minutum* and *T. platneri*, but it is identical for the two species (Stouthamer et al. 2000a). These difficulties, along with the possible presence of other species in release zones, complicate augmentative biological control efforts by requiring that both pre- and post-release assessment be dependent upon processes of specimen identification that are often not practical.

Using minor morphological differences, other species of *Trichogramma* associated with tortricid orchard pests can be recognized from members of the *T. minutum* complex (Pinto 1999), but these differences are confounded by an incomplete knowledge of intraspecific variation and apparent intermediacy. Problems of morphological intermediacy are especially apparent in the distinction between *T. platneri* and *T. californicum* Nagaraja and Nagarkatti, two species syntopic in western North America (Pinto 1999).

Trichogramma californicum was described from specimens reared from eggs of the Douglas fir tussock moth, *Orgyia pseudotsugata* (McDunnogh), collected from Alturas, Modoc County, in northeastern California (Nagaraja and Nagarkatti 1973). It was distinguished primarily by morphological features, but also because it was reproductively incompatible with laboratory cultures of other species of *Trichogramma* available at the time. *Trichogramma californicum* and *T. minutum* were distinguished by color, length and shape of flagelliform antennal setae, and ovipositor to hind tibia length ratio. The morphological distinctness of *T. californicum* was reassessed in a recent revision of the North American species of *Trichogramma* (Pinto 1999). No successful hybridization was found between the then available cultures of *T. californicum* and four other species: *T. exiguum* Pinto and Platner, *T. funestum* Pinto and Oatman, *T. interius* Pinto, and the Cow Head Lake (PCHL, Modoc Co., CA) culture of *T. platneri*, but it was noted that some crosses that should have been done had not yet been conducted (Pinto 1999). Recently, three cultures of *T. californicum*, CAAD, CASB, and CAYK (Table 1), were shown to be different from *T. minutum*, *T. platneri*, and two cultures of *T. exiguum* (EXHN, EXSL) at three loci using allozymic electrophoresis (Burks and Pinto 2002). High allozymic variability and low reproductive compatibility among the three cultures of *T. californicum* provided little evidence of their conspecificity, but

there was not enough evidence to exclude any of the three cultures from the rest of the species.

Trichogramma exiguum was described from eastern North America (Pinto et al. 1978). *Trichogramma exiguum* and *T. californicum* have similar male genitalic structure, and the flagelliform setae are relatively short and abruptly tapered in both (Pinto 1999). *Trichogramma exiguum* is also similar morphologically to the *T. minutum* complex, and is most likely to be confused with *T. minutum* because the two species are sympatric in eastern North America and are found on the same hosts, including codling moth and oriental fruit moth.

The purpose of this study is to investigate the potential of quantitative morphometric analysis to separate both males and females of the *T. minutum* complex, *T. californicum*, and *T. exiguum*.

MATERIALS AND METHODS

Specimens.—A total of 496 specimens were measured in this study, 231 males and 265 females, from 47 different laboratory-reared cultures (Table 1). Each culture originated from a single mated female that emerged from a field-collected host egg. Cultures were maintained in the laboratory at 21–27° C on irradiated *Trichoplusia ni* (Hübner) eggs. Selection of specimens was conducted on a culture by culture basis, with each included culture having been identified through complete direct or indirect reproductive compatibility with a reference culture (MCVA for *T. minutum*, PRV1 for *T. platneri*, CAAD for *T. californicum*, and EXSL for *T. exiguum*) and by morphological characteristics reported in Pinto (1999). Cultures for which reproductive compatibility data were not available were used only in posterior tests of the discriminant functions (indicated by asterisk in Table 1). All specimens were slide-mounted dorsoventrally in Canada Balsam (458 specimens) or Hoyer's medium (38 specimens) using uniform methodology (Platner et al. 1999). Specimens are stored in the University of California, Riverside, Department of Entomology Research Museum, each identified with an individual reference code UCRC ENT 43346–43841 and the voucher code RB1. Only specimens for which all measurements could be made unambiguously were included.

Characters.—A total of 27 morphological features for males and 26 for females were measured (Table 2, Fig. 1). All terms are the same as in Pinto (1999). Characters were selected on the basis of perceived taxonomic potential and presence of consistent landmarks (*sensu* Bookstein et al. 1985). Features that could not be accurately measured as a straight line were not used, with the exception of the longest flagelliform antennal seta length in males (lfs), which was represented as the sum of two measurements extending from the point of greatest curvature of the seta to its tip and base. We regard all of the landmarks to be readily placed, although perhaps the features of the antenna in both sexes could be the most easily confused. For clarity, these are illustrated in greater detail (Fig. 2). For cla, landmark 24 is the most apical point of the club, not counting the multiporus plate or basiconic peg sensilla, which may extend beyond the claval apex. In males, landmarks 25, 26, and 27 are based upon an earlier description of flagellar regions in male *Trichogramma* by Vincent and Goodpasture (1986), used again by Pinto (1999). The limits of each flagellomere are marked by distinct ventral constrictions (Fig. 2) that are proposed as homologous to separations between segments in species with more distinct flagellar segments (such as in the subgenera *Vanlisus* Pinto and *Trichogrammatza* Carver). In females, inclusion of measurements of the funicular segments (characters lfa-lfd and wfa-wfd) achieved a 2% reduction of the overall error in linear discriminant reclassification. However, these measurements were excluded from the final analysis because these landmark points were

Table 1. Collection details, code, and number of specimens studied for each culture. Unmarked cultures were included in the calibration dataset. Cultures marked by an asterisk (*) were included in the test dataset only.

Collection locality	Code	Collection date	# Of specimens	
			Male	Female
T. californicum	—	—	42	36
CA: Adin,	CAAD	24.vii.1992	10	10
CA: Alturas (types)*	CAAL	20.vi.1967	1	1
*CA, Garberville	CAGB	4.vi.1987	5	2
CA: Magee Mtn.	CAMM	12.vii.1992	2	3
CA: Sage*	CASG	11.iii.1980	2	0
CA: San Bernardino Mtns	CASB	12.viii.1997	10	10
ID: Greenleaf*	CAGL	13.viii.1999	2	0
WA: Yakima	CAYK	3–7.vii.1997	10	10
T. exiguum	—	—	19	15
AL: Selma	EXSL	5.x.1972	9	5
NC: Hendersonville	EXHN	15.viii.1997	10	10
T. minutum	—	—	102	122
CA: Chula Vista	MCVA	3.x.1973	10	10
CO: Colbran	MCLB	14.viii.1997	5	1
CO: Fruita	MFRU	13.viii.1997	4	6
ID: Bonner's Ferry	MBFY	27.viii.1997	3	7
KY: Rich Hill	MRHH	8.viii.1998	3	6
MD: Smithsburg	MSMT	7.viii.1998	5	5
ME: Winterville	MWTV	24.ix.1980	10	2
MN: southeastern	MSMN	2.viii.1992	5	3
MN: St. Paul	MSPL	21.i.1986	1	8
MO: Bigspring	MBGS	15.ix.1970	5	8
MO: Kirkwood	MKKW	24.ix.1970	0	4
NC: Hendersonville	MHND	26.viii.1997	5	7
NM: Albuquerque	MABQ	1.ix.1987	3	5
ON: Dryden	MDRY	25.vii.1990	5	7
TN: Monteagle	MONT	28.vi.1986	3	2
UT: Fairfield	MFFD	12.viii.1997	5	6
UT: Kanab	MKNB	7–10.viii.1997	3	9
UT: Springdale	MSGD	7.viii.1997	4	2
UT: Tropic	MTRP	8.viii.1997	5	8
WA: Mead	MEAD	21.viii.1997	9	6
WA: Wenatchee	MWEN	25.viii.1997	5	4
WI: Madison	MMDS	28–29.vii.1998	4	6
T. platneri	—	—	68	92
BC: Summerland	PSUM	1.viii.1997	4	6
CA: Boulder Creek	PBCK	11–12.ix.1997	5	6
CA: Cow Head Lake	PCHL	22.vii.1992	5	5
CA: El Toro	PELT	18.ii.1983	1	4
CA: Garberville	PGRB	5.vi.1987	5	3
CA: James Reserve	PJRV	7.vi.1985	5	8
CA: Julian	PJUL	21–23.vii.1998	1	4
CA: Newcastle	PNWC	6.ix.1990	5	1
CA: Riverside	PRVI	15.vii.1971	10	10
CA: Winters	PWTS	1.vii.1981	5	10
MT: Libby	PLIB	27.viii.1997	5	9
OR: Pendleton	PPND	24.viii.1997	5	10
WA: Colville	PCLV	22.viii.1997	5	5
WA: Granger	PGRN	24.viii.1987	5	5
WA: Walla Walla	PWWL	23.viii.1997	3	6
Total all species	—	—	231	265

Table 2. Characters measured and their descriptions. Landmarks refer to points in Figure 1.

Code	Sex	Landmarks	Description
Genitalia			
aed	M	2–13	Length of aedeagus from apodemes to tip (tips of both apodemes usually not equidistant from tip of aedeagus, so their position was averaged by drawing a line between tips apodemes and measuring from middle of that line)
lgc	M	1–13	Length of genital capsule (apical point determined by drawing line between tips of parameres and measuring from middle of that line)
wgc	M	3–4	Greatest width of genital capsule (without landmarks)
gcj	M	5–6	Width of genital capsule at base of dorsal lamina
apd	M	10–12	Apical distance (apical coordinate determined as with lgc)
lda	M	1–9	Length of dorsal aperture
ldl	M	9–11	Length of posterior extension of dorsal lamina
wdl	M	7–8	Width of dorsal lamina at the widest point of its shoulders
ivp	M	14–15	Length of intervolsellar process
ovp	F	16–20	Length of ovipositor sheaths from base of 1st and 2nd valvulae (= 1st and 2nd gonapophyses) to tip of 3rd valvula (= gonoplac)
svf	F	17–18	Length of 2nd valvifer (= 2nd gonocoxa) from base to medial corner of 3rd valvula
dtv	F	19–20	Lateral length of 3rd valvula from base to tip
ltv	F	18–20	Medial length of 3rd valvula from base to tip
wtv	F	18–19	Width of 3rd valvula base from medial corner to lateral corner
Antenna			
cla	M	24–25	Ventral length of 4th flagellar region
clb	M	25–26	Ventral length of 3rd flagellar region
clc	M	26–27	Ventral length of 2nd flagellar region
cld	M	27–28	Ventral length of 1st flagellar region
lcv	F	29–30	Maximum length of club
lfa	F	32–34	Ventral length of 2nd funicular segment
lfb	F	31–33	Dorsal length of 2nd funicular segment
lfc	F	36–38	Ventral length of 1st funicular segment
lfd	F	35–37	Dorsal length of 1st funicular segment
lfs	M	21–22–23	Longest flagelliform antennal seta length (flagelliform setae are almost always strongly arched, so measurements were taken by combining the lengths of lines projecting from the point of greatest curvature to the tip and base of the seta, respectively)
sca	M/F	39–40	Dorsal length of scape, from dorso-apical corner to dorso-apical corner of radicle
wfa	F	31–32	Apical width of 2nd funicular segment
wfb	F	33–34	Basal width of 2nd funicular segment
wfc	F	35–36	Apical width of 1st funicular segment
wfd	F	37–38	Basal width of 1st funicular segment
Legs			
lmb	M/F	41–42	Dorsal length of metatibia
mta	M/F	43–44	Dorsal length of basal metatarsal segment
mtb	M/F	45–46	Dorsal length of middle metatarsal segment
mtc	M/F	47–48	Dorsal length of apical metatarsal segment
spa	M/F	49–50	Length of metatibial spur, from socket to tip
spb	M/F	51–52	Length of mesotibial spur, from socket to tip
Wings			
lwg	M/F	57–58	Length of fore wing from its base immediately distal to humeral plate to where the M setal track reaches the wing margin
wwg	M/F	59–60	Width of fore wing from end of 3rd setal track to end of posterior-most setal track
lsv	M/F	53–54	Length of stigmal vein (the base of the stigmal vein cannot be determined using landmarks, and the pigmented apex of the stigmal vein could not always be determined with satisfactory accuracy, so the sockets of two consistently locatable setae were used as landmarks)
wsv	M/F	55–56	Width of stigma vein at its narrowest point, no landmarks
lfl	M/F	61–62	Length of longest marginal fringe seta of fore wing
lhm	M/F	63–64	Length of longest marginal fringe seta of hind wing

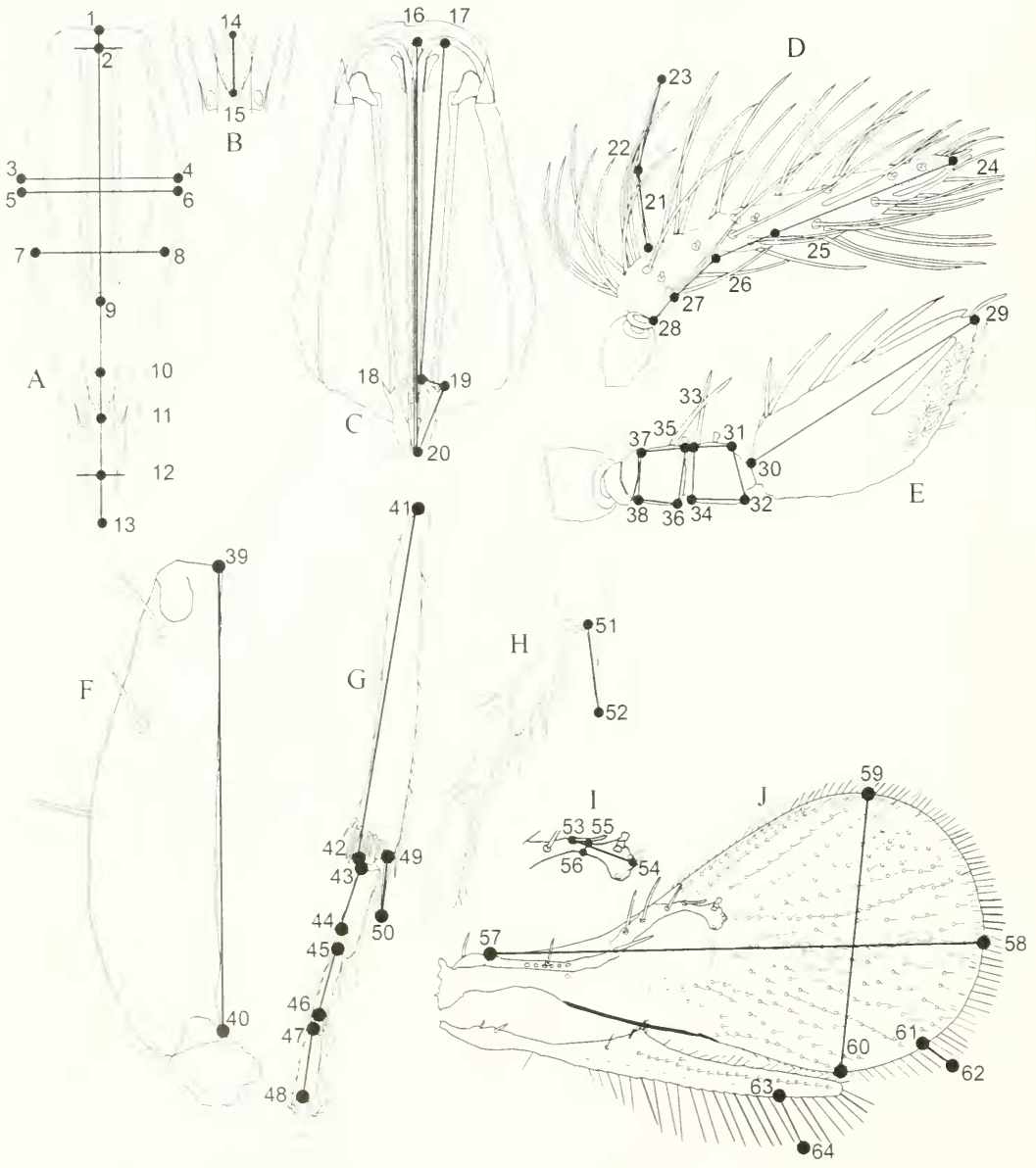

Fig. 1. Measurements for males and females of *Trichogramma*; numbers represent character landmarks defined in Table 2. A, Male genitalia. B, Intervolsellar process. C, Ovipositor. D, Male antenna. E, Female antenna. F, Scape. G, Metatibia and metatarsus. H, Mesotarsus. I, Stigmal vein. J, Wings.

difficult to position on the rounded edges of the segments, making it difficult to define them in an objective manner.

Landmark points could be readily observed in all of the specimens used for this analysis, and admittedly only good quality mounts can be scored for all of the relevant landmarks. The identification key provided in the discussion will be useful for most specimens, but in cases that require definitive results, a set of well-mounted specimens is necessary. This is achievable using the methods provided by Platner et al. (1999), which made possible

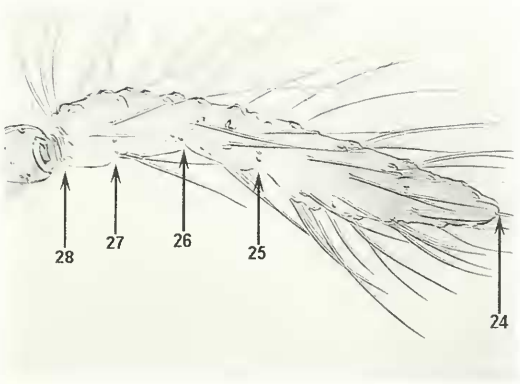

Fig. 2. Photograph of slide-mounted antenna of male *Trichogramma platneri* (PLIB) with landmarks indicated; numbers represent points defined in Table 2.

the use of nearly 500 uniformly positioned specimens for this study.

Measurements.—Specimens were measured using a Leica DMRB microscope through a Sony DXC-107 videochip camera using the same methods described in Heraty and Polaszek (2000). The computer program Morphosys (Meachum and Duncan 1987) was used to measure pixel distances between on-screen point coordinates and convert them to millimeter distances. Landmarks were gathered at magnifications of 200–640×. Linear measurements were analyzed using SAS release 8.00 software.

Statistical analyses.—Unless stated otherwise, all analyses were conducted on two calibration datasets, one for each sex (Table 1). Using the same specimen data, subset analyses comparing only a pair of species, or comparing the *T. minutum* complex as a whole with *T. californicum* or *T. exiguum*, were performed using principal component analysis, using the specimens of those species contained in the calibration datasets. These data sets are available in SAS format files from the corresponding author upon request.

Males and females of the four species were analyzed separately in an 'all species' calibration dataset (ASD) using 19 variables, although not all variables were shared in each dataset (Table 6). Males were studied further in four subset analyses that focused on comparisons of *T. minutum* with *T. platneri*, *T. californicum* with *T. exiguum*, and the *T. minutum* complex with *T. californicum* and *T. exiguum*. In each case the morphometric variables were a subset of the variables used in the ASD of males (Table 6). Females of *T. minutum* and *T. platneri* were analyzed separately using a subset of 12 variables from the ASD of females. Variables for each analysis were chosen using stepwise discriminant analysis based on a covariance matrix using species as the class variable (Table 6). The significance level for inclusion of variables was 0.15 as determined by a multivariate F-test.

Principal component analysis: Principal component analyses were conducted using variance-covariance matrices for the included variables. Raw and transformed logarithmic (base 10), and logarithmic (base e) data were investigated (Marcus 1990), but only the results obtained from the logarithmic (base e) transformed data are presented. Those data were chosen because they exhibited the smallest magnitude and number of departures from normality for variables within each species. Principal component analyses of males that compared pairs of species produced different results that were sometimes diagnostic when results from the complete analysis were less or not at all diagnostic. For this reason, subset analyses comparing each pair of species were performed. The specimens for each subset analysis consisted of all the specimens of the pair of species examined from the males calibration dataset. The *T. minutum* complex was treated as a single unit in subset analyses. Subset analyses of females did not show additional resolution, and only the complete analysis of females is reported.

Canonical variate analysis: Canonical variate analyses were conducted using raw data only. Subset analyses were conducted using various combinations of spe-

cies, but failed to show additional resolution, and are not presented.

The absolute value of standardized canonical coefficients can be used to determine the contribution of a variable to a canonical variate (Umphrey 1996). This process is not as simple as analyzing the loadings of variables into principal components (Reyment 1990), and conclusions based on these values must be made very carefully, if any are made at all (Woolley et al. 1994). However, these values were analyzed in this study with the caveat that conclusions more specific than an assessment of contribution to a variate cannot be made with confidence.

Testing: Test datasets containing cultures of *T. californicum* for which reproductive compatibility data were not available were analyzed using discriminant functions generated from the calibration datasets.

Resampling: Canonical variate analysis, and discriminant analyses in general, have been found to introduce bias for class separation such that they can produce results that are not robust to testing with new observations (Lance et al. 2000). In order to test for replicability, an original variation of the standard jackknife resampling method was used for each canonical variate analysis. This consisted of removing all observations of a culture from the original dataset, with the remaining observations becoming a new calibration dataset, while the removed observations were then used as a test dataset and classified using the linear discriminant function generated from the modified calibration dataset. This was repeated for each culture. This resampling method was chosen to illustrate the recommended method of using the linear discriminant function to classify unknown specimens, and to provide an expected classification error rate per culture for that method.

Discriminant analysis test class: Males and females from five *T. californicum* cultures were analyzed only as a test class for the calibration datasets because conspecificity with other cultures of *T. californicum* could not be verified through reproductive compatibility. These specimens were used to test the discriminant functions derived from the calibration datasets. Test classes using specimens from the same cultures comprising the calibration dataset were not made as this would violate the assumption of independent sampling, leading to unrealistically optimistic results.

RESULTS

Univariate and bivariate analyses.—Tables 3 and 4 list the mean and standard deviation of each character by species for the respective sexes in the calibration data sets. Table 5 lists the ranges of ratios discussed below. There was a pattern of overall size difference between the species in males, in the order of *T. platneri* > *T. minutum* > *T. californicum* > *T. exiguum*. The trend is similar in females, except that specimens of *T. minutum* were slightly larger than *T. platneri*. In no case could males or females of any species be separated without overlap using univariate or bivariate measures. Males of *T. californicum* and *T. exiguum* could be separated from those of the *T. minutum* complex using the length of the longest flagelliform seta (lfs) alone, with minor overlap (Table 3, Fig. 3A). In *T. californicum* and *T. exiguum*, this seta was 0.07 mm or less except for two large specimens of *T. californicum* (lfs = 0.071 mm and 0.073 mm), while that in the *T. minutum* complex was 0.071 mm or greater except for two small specimens of *T. platneri* (lfs = 0.063 mm and 0.068 mm). The size of the specimens is best indicated by the correlated length of the metatibia, which was 0.180 and 0.196 mm in the same specimens of *T. californicum* and 0.125 and 0.158 mm in the two specimens of *T. platneri*.

Males of *T. californicum* can be separated from *T. exiguum* using a ratio of stigmal

Table 3. Univariate statistics for male characters. Means on top with standard deviations in parentheses, and range below. All units in millimeters. Character abbreviations explained in Table 2.

Character	*T. californicum*	*T. exiguum*	*T. minutum*	*T. platneri*	Average
aed	0.136 (0.013)	0.114 (0.012)	0.151 (0.016)	0.153 (0.018)	0.146 (0.019)
	0.104–0.163	0.099–0.145	0.115–0.186	0.112–0.183	
apd	0.035 (0.003)	0.025 (0.002)	0.036 (0.003)	0.036 (0.003)	0.035 (0.004)
	0.028–0.045	0.020–0.030	0.030–0.044	0.028–0.043	
cla	0.091 (0.012)	0.087 (0.011)	0.107 (0.013)	0.108 (0.013)	0.103 (0.014)
	0.060–0.113	0.067–0.110	0.076–0.138	0.079–0.136	
clb	0.029 (0.004)	0.027 (0.006)	0.033 (0.006)	0.033 (0.006)	0.032 (0.006)
	0.020–0.038	0.018–0.040	0.018–0.045	0.018–0.052	
clc	0.026 (0.005)	0.026 (0.006)	0.031 (0.004)	0.030 (0.005)	0.030 (0.005)
	0.015–0.035	0.015–0.039	0.019–0.041	0.020–0.039	
cld	0.019 (0.003)	0.018 (0.003)	0.022 (0.003)	0.022 (0.003)	0.021 (0.003)
	0.014–0.025	0.011–0.023	0.016–0.031	0.014–0.032	
gcj	0.046 (0.004)	0.041 (0.004)	0.046 (0.005)	0.050 (0.006)	0.047 (0.006)
	0.037–0.055	0.032–0.049	0.036–0.059	0.037–0.064	
ivp	0.038 (0.005)	0.034 (0.004)	0.043 (0.004)	0.024 (0.004)	0.041 (0.005)
	0.028–0.045	0.027–0.041	0.034–0.052	0.029–0.052	
lda	0.080 (0.008)	0.066 (0.009)	0.086 (0.010)	0.093 (0.012)	0.086 (0.013)
	0.058–0.096	0.055–0.093	0.062–0.109	0.069–0.114	
ldl	0.014 (0.002)	0.011 (0.002)	0.016 (0.002)	0.016 (0.002)	0.015 (0.002)
	0.012–0.018	0.009–0.014	0.013–0.021	0.012–0.022	
lfl	0.036 (0.003)	0.032 (0.004)	0.036 (0.004)	0.034 (0.003)	0.035 (0.004)
	0.029–0.043	0.025–0.040	0.026–0.046	0.027–0.043	
lfs	0.063 (0.006)	0.060 (0.005)	0.086 (0.006)	0.080 (0.007)	0.079 (0.011)
	0.051–0.076	0.050–0.069	0.073–0.102	0.059–0.098	
lgc	0.133 (0.012)	0.112 (0.010)	0.146 (0.014)	0.150 (0.016)	0.142 (0.018)
	0.105–0.158	0.099–0.140	0.116–0.175	0.114–0.177	
lhm	0.066 (0.005)	0.056 (0.006)	0.064 (0.006)	0.066 (0.005)	0.064 (0.006)
	0.055–0.079	0.043–0.069	0.051–0.079	0.054–0.080	
lmb	0.167 (0.017)	0.149 (0.021)	0.178 (0.024)	0.183 (0.025)	0.176 (0.025)
	0.130–0.201	0.114–0.203	0.127–0.237	0.125–0.228	
lsv	0.041 (0.004)	0.042 (0.005)	0.047 (0.006)	0.047 (0.005)	0.046 (0.006)
	0.034–0.051	0.034–0.057	0.033–0.063	0.037–0.062	
lwg	0.489 (0.044)	0.433 (0.050)	0.522 (0.060)	0.541 (0.060)	0.516 (0.064)
	0.373–0.592	0.342–0.558	0.384–0.654	0.394–0.685	
mta	0.035 (0.005)	0.030 (0.005)	0.034 (0.005)	0.035 (0.005)	0.034 (0.005)
	0.024–0.044	0.024–0.041	0.023–0.049	0.022–0.045	
mtb	0.039 (0.005)	0.033 (0.004)	0.039 (0.006)	0.040 (0.006)	0.039 (0.006)
	0.025–0.048	0.023–0.041	0.025–0.053	0.024–0.055	
mtc	0.031 (0.003)	0.029 (0.003)	0.033 (0.003)	0.033 (0.003)	0.032 (0.003)
	0.026–0.037	0.024–0.037	0.025–0.041	0.027–0.041	
sca	0.078 (0.009)	0.072 (0.010)	0.085 (0.009)	0.085 (0.010)	0.083 (0.010)
	0.060–0.095	0.052–0.093	0.066–0.110	0.063–0.110	
spa	0.026 (0.003)	0.024 (0.003)	0.029 (0.003)	0.030 (0.003)	0.028 (0.004)
	0.020–0.033	0.017–0.033	0.023–0.036	0.021–0.037	
spb	0.029 (0.005)	0.029 (0.004)	0.033 (0.004)	0.035 (0.004)	0.032 (0.005)
	0.018–0.038	0.023–0.039	0.023–0.041	0.023–0.044	
wdl	0.037 (0.003)	0.036 (0.003)	0.041 (0.004)	0.042 (0.005)	0.040 (0.005)
	0.030–0.042	0.032–0.042	0.032–0.051	0.030–0.053	
wgc	0.047 (0.004)	0.042 (0.005)	0.049 (0.005)	0.051 (0.007)	0.048 (0.006)
	0.038–0.056	0.034–0.051	0.037–0.061	0.037–0.064	
wsv	0.006 (0.001)	0.005 (0.001)	0.005 (0.001)	0.006 (0.002)	0.006 (0.001)
	0.003–0.009	0.004–0.007	0.003–0.008	0.004–0.011	
wwg	0.279 (0.029)	0.239 (0.030)	0.291 (0.036)	0.309 (0.039)	0.290 (0.040)
	0.202–0.349	0.187–0.315	0.208–0.373	0.222–0.424	

Table 4. Univariate statistics for female characters. Means on top with standard deviations in parentheses, and range below. All units in millimeters. Character abbreviations explained in Table 2.

Character	*T. californicum*	*T. exiguum*	*T. minutum*	*T. platneri*	Average
dtv	0.038 (0.003)	0.034 (0.004)	0.037 (0.004)	0.034 (0.004)	0.036 (0.004)
	0.033–0.043	0.027–0.040	0.026–0.050	0.026–0.042	
lcv	0.089 (0.008)	0.084 (0.010)	0.087 (0.009)	0.086 (0.009)	0.087 (0.009)
	0.073–0.102	0.066–0.098	0.069–0.106	0.067–0.105	
lfa	0.016 (0.003)	0.015 (0.002)	0.017 (0.003)	0.017 (0.003)	0.016 (0.003)
	0.012–0.023	0.011–0.019	0.011–0.026	0.010–0.024	
lfb	0.013 (0.002)	0.011 (0.002)	0.013 (0.002)	0.013 (0.003)	0.013 (0.002)
	0.010–0.017	0.008–0.015	0.006–0.020	0.008–0.020	
lfc	0.015 (0.003)	0.012 (0.002)	0.013 (0.002)	0.013 (0.002)	0.013 (0.002)
	0.009–0.022	0.010–0.016	0.007–0.022	0.009–0.019	
lfd	0.015 (0.003)	0.013 (0.003)	0.013 (0.002)	0.013 (0.002)	0.013 (0.003)
	0.010–0.021	0.007–0.018	0.007–0.019	0.008–0.019	
lfl	0.034 (0.003)	0.030 (0.002)	0.035 (0.004)	0.034 (0.003)	0.034 (0.004)
	0.029–0.043	0.027–0.036	0.026–0.046	0.022–0.044	
lhm	0.065 (0.010)	0.059 (0.004)	0.064 (0.006)	0.067 (0.007)	0.065 (0.007)
	0.051–0.085	0.052–0.065	0.048–0.081	0.050–0.082	
lsv	0.041 (0.005)	0.044 (0.007)	0.045 (0.006)	0.045 (0.006)	0.044 (0.006)
	0.031–0.050	0.035–0.061	0.032–0.060	0.033–0.062	
ltv	0.037 (0.003)	0.035 (0.004)	0.037 (0.004)	0.035 (0.004)	0.036 (0.004)
	0.032–0.042	0.029–0.040	0.026–0.048	0.025–0.043	
lwg	0.519 (0.062)	0.463 (0.064)	0.516 (0.067)	0.532 (0.071)	0.519 (0.070)
	0.406–0.651	0.379–0.571	0.373–0.661	0.381–0.670	
mta	0.046 (0.007)	0.037 (0.006)	0.042 (0.007)	0.042 (0.007)	0.042 (0.007)
	0.032–0.062	0.028–0.047	0.027–0.059	0.026–0.061	
mtb	0.048 (0.005)	0.040 (0.004)	0.046 (0.007)	0.046 (0.007)	0.046 (0.007)
	0.037–0.058	0.032–0.044	0.031–0.062	0.028–0.059	
mtc	0.035 (0.004)	0.032 (0.003)	0.034 (0.004)	0.034 (0.004)	0.034 (0.004)
	0.028–0.043	0.027–0.038	0.026–0.044	0.025–0.045	
ovp	0.185 (0.018)	0.180 (0.024)	0.201 (0.023)	0.195 (0.025)	0.196 (0.024)
	0.152–0.222	0.143–0.218	0.145–0.245	0.136–0.233	
sca	0.096 (0.011)	0.090 (0.012)	0.098 (0.012)	0.098 (0.012)	0.097 (0.012)
	0.075–0.122	0.072–0.110	0.068–0.124	0.072–0.124	
spa	0.027 (0.002)	0.024 (0.004)	0.028 (0.003)	0.029 (0.003)	0.028 (0.003)
	0.023–0.033	0.017–0.028	0.022–0.037	0.019–0.036	
spb	0.033 (0.005)	0.031 (0.005)	0.033 (0.004)	0.034 (0.004)	0.034 (0.005)
	0.024–0.043	0.021–0.038	0.023–0.043	0.022–0.046	
svf	0.151 (0.016)	0.146 (0.021)	0.164 (0.020)	0.162 (0.022)	0.161 (0.021)
	0.119–0.183	0.116–0.181	0.113–0.205	0.108–0.201	
wfa	0.016 (0.002)	0.017 (0.002)	0.017 (0.002)	0.017 (0.002)	0.017 (0.002)
	0.013–0.021	0.013–0.022	0.014–0.021	0.010–0.022	
wfb	0.013 (0.001)	0.014 (0.002)	0.015 (0.001)	0.014 (0.002)	0.014 (0.002)
	0.010–0.016	0.011–0.018	0.011–0.018	0.011–0.019	
wfc	0.016 (0.002)	0.017 (0.002)	0.016 (0.001)	0.016 (0.002)	0.016 (0.002)
	0.012–0.019	0.013–0.020	0.013–0.020	0.012–0.020	
wfd	0.013 (0.002)	0.014 (0.002)	0.014 (0.001)	0.014 (0.001)	0.014 (0.001)
	0.011–0.017	0.011–0.016	0.012–0.018	0.011–0.019	
wsv	0.007 (0.001)	0.005 (0.001)	0.005 (0.001)	0.006 (0.001)	0.006 (0.001)
	0.004–0.009	0.004–0.007	0.004–0.009	0.004–0.010	
wtv	0.011 (0.002)	0.010 (0.002)	0.011 (0.002)	0.010 (0.002)	0.011 (0.002)
	0.007–0.014	0.007–0.013	0.006–0.015	0.007–0.014	
wwg	0.287 (0.040)	0.248 (0.038)	0.277 (0.041)	0.290 (0.046)	0.281 (0.043)
	0.216–0.370	0.197–0.314	0.192–0.376	0.195–0.382	

Table 5. Ranges of ratios plotted in Figs. 3A–3E.

Ratio	*T. californicum*	*T. exiguum*	*T. minutum*	*T. platneri*
lfs/wwg	0.59–0.73	0.66–0.80	0.60–0.87	0.57–0.78
lsv/apd	0.79–1.38	1.36–1.93	0.97–1.71	1.06–1.58
ovp/mtb	3.42–4.26	4.06–5.01	3.62–5.27	3.66–5.80
ovp/wwg	0.59–0.73	0.66–0.80	0.60–0.87	0.57–0.78
lfs/lge	0.22–0.35	0.24–0.40	0.19–0.35	0.17–0.38

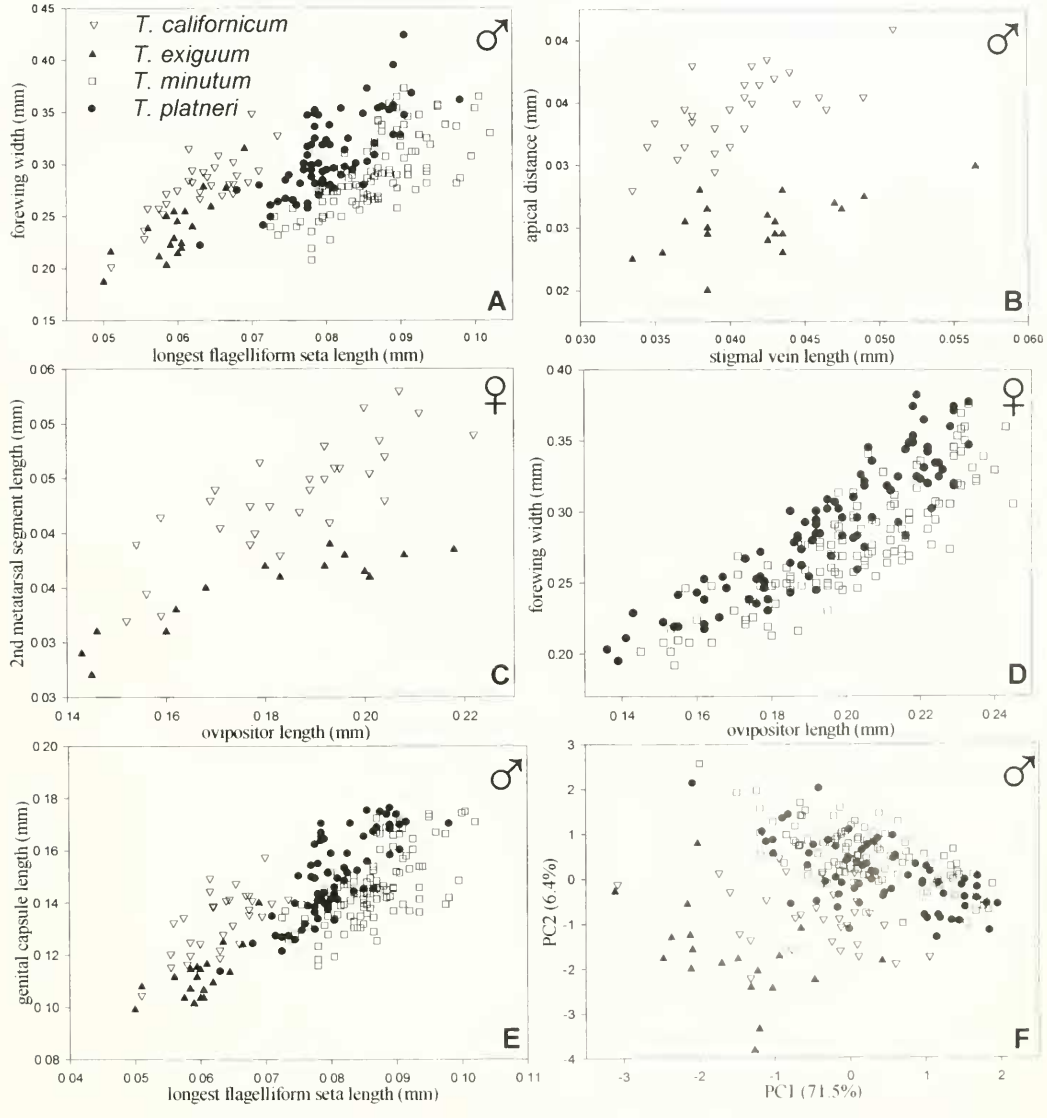

Fig. 3. A–E. Bivariate scatterplots. A, Longest flagelliform seta length vs. forewing width, all species. B, Stigmal vein length vs. apical distance, *T. californicum* and *T. exiguum*. C, Ovipositor length vs. 2nd metatarsal segment length, *T. californicum* and *T. exiguum*. D, Ovipositor length vs. forewing width, *T. minutum* and *T. platneri*. E, Longest flagelliform seta length vs. genital capsule length, all species. F, First two principal components of the male calibration data set, proportion of sample variance in parentheses.

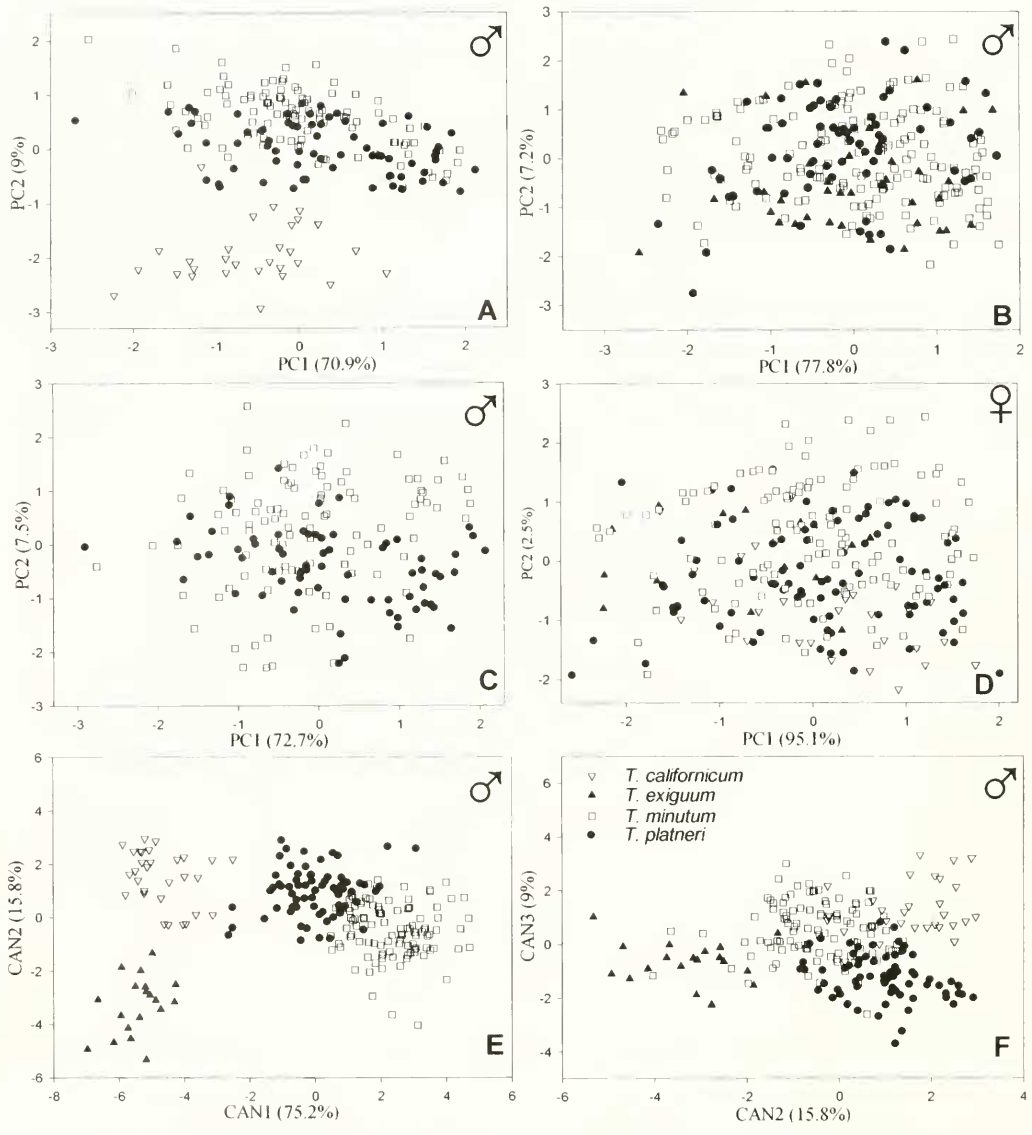

Fig. 4. Plots of first two principal components of selected analyses, proportion of sample variance in parentheses. A, Subset analysis of *T. californicum* vs. *T. minutum* complex males. B, Subset analysis of *T. exiguum* vs. *T. minutum* complex males. C, Subset analysis of *T. minutum* vs. *T. platneri* males. D, Female calibration data set. E–F. Plots of selected canonical variates for males.

vein length (lsv) and apical distance of the genital capsule as measured from the base of the intervolsellar process to the apex of the parameres (apd) (Table 5, Fig. 3B) with overlap involving only one small specimen of *T. exiguum*. In *T. californicum*, the ratio of lsv/apd was less than 1.40, while it was greater than 1.45 in *T. exiguum* except for the single unusual specimen (lsv/apd = 1.36; metatibial length = 0.135 mm).

Males of *T. minutum* and *T. platneri* could be partially separated using a ratio of length of the longest flagelliform antennal seta to fore wing width (wwg) (Fig. 3A). In 79% of *T. minutum* males, this ratio

Table 6. Variables selected for multivariate comparisons using stepwise discriminant analysis, listed in descending order of final F value. Bolded variables were selected in four or more separate analyses of males.

Comparison	Variables	Total # of variables/27
Males		
All species	**lfs, apd, wdl, wwg, gcj,** mta, **lda,** spb, mtb, cla, lmb, wgc, wsv, sca, ivp, lgc, aed, mtc, lsv	19
T. californicum vs. *T. minutum* complex	**lfs,** mta, wgc, mtb, **apd,** wsv, lgc, cla, **wdl,** ldl, mtc, **lda**	12
T. californicum vs. *T. exiguum*	lgc, lsv, sca, **wwg, spb,** lhm, clc, **gcj**	8
T. exiguum vs. *T. minutum* complex	**lfs, lda,** lmb, **apd,** ivp, **spb, gcj,** mta, **wwg, wdl**	10
T. minutum vs. *T. platneri*	**gcj, lfs, wwg,** wgc, **lda, wdl,** sca, lfl, aed, **apd,** ivp, mtb, **spb**	13
Females		
All species	lsv, lmb, lfl, ovp, lcv, dtv, mta, ltv, spa, mtb, svf, lwg, mtc, **wwg,** wsv, lhm	16
T. minutum vs. *T. platneri*	lfl, lsv, lcv, ovp, **wwg,** dtv, svf, **spb,** lwg, lhm	10

was greater than 0.28, while it was 0.28 or less in 78% of *T. platneri* males.

In no case could females of any species be completely separated using univariate or bivariate measures, but partial segregation could be found here in ratios involving ovipositor length (ovp). Over 90% of *T. californicum* specimens had a ratio of ovipositor length to 2nd metatarsal segment length (mtb) of less than 4.25, while in 80% of *T. exiguum* specimens the ratio was greater than 4.25 (Table 5, Fig. 3C). The ratio of ovipositor length to fore wing width (wwg) provided the best bivariate separation of *T. minutum* and *T. platneri* females (Fig. 3D), although because of the considerable overlap this would not be a useful characteristic for identification (Table 5). In 70% of *T. platneri* specimens, ovp/wwg was less than 0.7, while it was greater than 0.7 in 70% of *T. minutum* specimens.

Principal component analysis.—The complete analysis of males output no components with strong diagnostic power, but some rough groupings of species were apparent (Fig. 3F). Complete separation was obtained between *T. exiguum* and the *T. minutum* complex based on the first and second components. Partial separation was obtained between *T. californicum* and the *T. minutum* complex based on those same components, with most of the overlap involving *T. platneri.* The first component appeared to be strongly size correlated with all loadings large and positive (Table 7). Longest flagelliform seta length (lfs) loaded strongly onto the second component (Table 7). The influence of this character is consistent with the findings of the univariate analyses. Although the remaining components in each analysis contained a significant proportion of the variance, they had no more diagnostic value than the first two components and are not reported.

Subset analyses: In the analysis comparing *T. californicum* and *T. minutum* complex males only, the second component (Fig. 4A) could be used to separate species, with only one exception, a *T. californicum* specimen from CAYK. All other *T. californicum* specimens were scored at −1 or less on this component, while all *T. minutum* complex specimens were scored at −0.924 or above. The only variable with a high loading on this component was longest flagelliform seta length, which is con-

Table 7. Eigenvalues and loadings for the first two principal components of the covariance matrix of the log$_{10}$ transformed male and female calibration datasets for all species, and the subset analyses of *T. minutum* vs *T. platneri*, the *T. minutum* complex vs *T. californicum*, and the *T. minutum* complex vs *T. exiguum*. Variables selected by stepwise discriminant analysis (Table 6).

	All species, males		*T. minutum* vs *T. platneri*		*T. minutum* complex vs *T. californicum*		*T. minutum* complex vs *T. exiguum*			All species, females	
Variable	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	Variable	PC1	PC2
Eigenvalue	0.216	0.018	0.123	0.013	0.137	0.709	0.148	0.779	Eigenvalue	0.211	0.034
Proportion	0.715	0.064	0.727	0.075	0.709	0.090	0.778	0.072	Proportion	0.690	0.112
aed	0.95	0.05	0.97	−0.10	—	—	—	—	dtv	0.69	0.24
apd	0.83	−0.09	0.84	0.14	—	—	0.86	0.36	lcv	0.92	0.07
cla	0.87	0.28	—	—	0.88	0.27	—	—	lfl	−0.02	0.09
gcj	0.88	−0.20	0.92	−0.27	0.88	−0.17	0.90	−0.21	lhm	0.74	0.01
ivp	—	—	0.66	−0.07	—	—	0.74	0.37	lmb	0.97	0.03
lda	0.93	−0.07	0.94	−0.13	—	—	0.95	−0.04	lsv	0.77	0.13
ldl	0.73	0.09	—	—	0.74	0.15	—	—	ltv	0.75	0.22
lfl	0.01	0.01	−0.01	0.80	—	—	—	—	lwg	0.98	0.01
lfs	0.63	0.70	0.61	0.36	0.63	0.68	0.76	0.57	mta	0.93	0.03
lgc	0.96	0.03	—	—	0.95	0.05	—	—	mtb	0.92	0.10
lhm	0.63	−0.34	—	—	0.63	−0.37	—	—	mtc	0.90	0.01
lmb	0.97	−0.11	—	—	—	—	0.96	−0.14	ovp	0.93	0.21
lsv	0.78	0.26	—	—	—	—	—	—	spa	0.80	0.10
mta	0.85	−0.35	—	—	0.86	−0.37	0.88	−0.21	svf	0.93	0.18
mtb	0.86	−0.22	0.87	0.28	0.87	−0.24	—	—	wsv	0.50	−0.86
sca	0.94	0.01	0.93	0.10	—	—	—	—	wwg	0.96	−0.04
scb	0.86	0.19	0.84	0.14	0.87	0.19	—	—			
wdl	0.83	0.09	0.84	−0.14	0.83	0.13	0.86	−0.15			
wgc	0.91	−0.14	0.94	−0.23	0.90	−0.11	0.84	−0.16			
wwg	0.94	−0.12	0.94	0.09	0.94	−0.14	0.96	−0.11			

sistent with the univariate separation involving this variable (Table 3).

In the analysis comparing *T. exiguum* and *T. minutum* complex males, a complete separation is evident based on the first two components (Fig. 4B). The first component had high loadings of all variables, and is assumed to represent size. Longest flagelliform seta length loaded strongly onto the second component (Table 7).

The analysis involving only males of *T. minutum* and *T. platneri* output no components with strong diagnostic power (Fig. 4C). No component except for the first had high loadings of both longest flagelliform seta length and fore wing width, so the partial bivariate separation found using these variables was not upheld in this analysis.

In the principal component analysis of all females, no component or simple combination of components proved to have diagnostic value (Fig. 4D). Only the first component had high loadings of ovipositor length, 2nd metatarsal segment length, or fore wing width, implying that the partial segregations reported above using these variables were correlated with size. This in itself does not invalidate those patterns, because the first component may contain shape as well as size information (Marcus 1990).

Canonical variate analysis.—Males of all species could be discriminated with an overall error rate of 1.35% in the calibration dataset (Table 8). Most of the classification errors involved *T. minutum* and *T. platneri*, but one small specimen of *T. platneri* (metatibial length = 0.158 mm) was identified as *T. californicum*. This specimen was one of the two that overlapped with

Table 8. Results of linear discriminant reclassification of males using the canonical variate results, with error rates.

From	Number classified into species:					
	T. californicum	*T. exiguum*	*T. minutum*	*T. platneri*	Total	% Error
T. californicum	30	0	0	0	30	0
T. exiguum	0	19	0	0	19	0
T. minutum	0	0	98	4	102	3.92
T. platneri	1	0	0	67	68	1.47
Total	31	19	98	71	219	1.35

the grouping of *T. californicum* in the univariate comparison involving longest flagelliform seta length (lfs) (Fig. 3A). The best separation of *T. californicum* and *T. exiguum* from the *T. minutum* complex was found along the first canonical variate (Fig. 4E), with minor overlap between *T. californicum* and *T. platneri*. Longest flagelliform seta length (lfs), genital capsule width at base of dorsal lamina (gcj), and genital capsule width at its widest point (wgc) appeared to contribute the most to this variate according to their standardized canonical coefficient values (Table 9). The large contribution of lfs is consistent with the univariate separation of these species mentioned earlier, but the relation of the contribution of this variable to that of the genitalic characters is not clear. A plot of lfs and lgc (Fig. 3E) resembles the plot of the first two canonical variates, except that the clouds of points are not circularized as in plots of canonical variates. This similarity helps demonstrate the probable relationship between these raw variables and the canonical variate results.

Trichogramma exiguum was best separated from *T. californicum* and *T. platneri* along the second canonical variate (Figs 3E, 3F). Interpretation of the standardized

Table 9. Raw and standardized canonical coefficients for males.

Char.	Raw coefficients			Standardized coefficients		
	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
Constant	−14.70	−1.97	0.43	—	—	—
aed	38.49	−0.90	82.63	0.75	−0.02	1.60
apd	48.36	−198.70	288.18	0.21	−0.87	1.26
cla	19.38	31.92	−34.96	0.28	0.47	−0.51
gcj	−391.19	−197.72	−286.84	−2.35	−1.19	−1.73
lda	−24.21	−112.43	−118.33	−0.31	−1.42	−1.49
ldl	92.14	−101.29	−59.61	0.22	−0.25	−0.14
lfl	46.24	−8.76	63.29	0.17	−0.03	0.24
lfs	198.05	12.35	−11.67	2.26	0.14	−0.13
lgc	81.68	−10.89	−67.31	1.43	−0.19	−1.18
lhm	−35.83	5.77	−16.77	−0.27	0.04	−0.13
lmb	−24.26	66.63	19.43	−0.61	1.67	0.49
lsv	3.63	70.05	−45.05	0.02	0.43	−0.28
mta	−115.54	−8.43	112.11	−0.61	−0.04	0.59
mtb	−80.94	−77.57	51.38	−0.47	−0.45	0.30
sca	42.31	83.32	58.40	0.42	0.83	0.58
spb	−102.45	66.03	−95.80	−0.46	0.30	−0.43
wdl	88.38	243.56	−47.55	0.41	1.12	−0.22
wgc	187.24	116.12	284.56	1.13	0.70	1.71
wwg	−10.54	−45.62	−16.59	−0.42	−1.83	−0.66

Table 10. Results of linear discriminant reclassification of females using the canonical variate results, with error rates.

From	Number classified into species:					
	T. californicum	*T. exiguum*	*T. minutum*	*T. platneri*	Total	% Error
T. californicum	29	0	1	0	30	3.3
T. exiguum	0	13	1	1	15	13.3
T. minutum	2	2	99	19	122	18.9
T. platneri	3	2	11	76	92	17.4
Total	34	17	112	96	259	13.2

canonical coefficients of this variate cannot be made with confidence because of relatively large contributions of variables from many different body regions.

Trichogramma platneri and *T. exiguum* were weakly separated from *T. minutum* and *T. californicum* along the third canonical variate (Fig. 4F). The weak segregation along this variate, although of minor value taken alone, enhances the discriminatory power of the analysis when used in combination with the other variates. Six characters, all from the male genitalia, contributed strongly to this variate: aedeagus length (aed), apical distance (apd), genital capsule width at base of dorsal lamina (gcj), dorsal aperture length (lda), genital capsule length (lgc), and genital capsule width at widest point (wgc). All except aedeagus length describe the shape of the genital capsule.

In the analysis of the calibration dataset for females, specimens were identified with an overall error rate of 13.2%, mostly involving misidentification of *T. minutum* into *T. platneri* and vice versa (Table 10). *Trichogramma californicum* was weakly separated from the other species along the first canonical variate (Fig. 5A). The largest contribution to this variate was made by ovipositor length (ovp) (Table 11), but there were large contributions from metatibial length (lmb) and 2nd valvifer length (svf). It is likely that this represents part of the strong diagnostic power found using ratios involving ovipositor length, with other variables either correlating with it or correcting it for body size variation. *Trichogramma platneri* was weakly separated from the other species along the second canonical variate. This variable has little discriminatory value taken by itself, but it enhances the discriminatory power of the analysis when used in combination

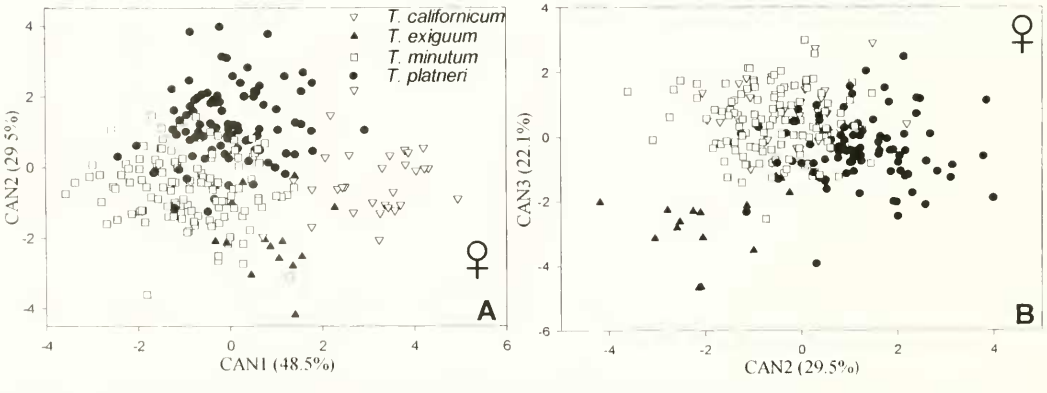

Fig. 5. A–B. Plots of selected canonical variates for females. Proportion of sample variance given in parentheses on axis labels.

Table 11. Raw and standardized canonical coefficients for females.

Char.	Raw coefficients			Standardized coefficients		
	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
Constant	7.62	7.58	9.02	—	—	—
dtv	56.90	−99.86	177.75	0.24	0.42	0.74
lcv	28.82	−154.06	−4.65	0.26	−1.38	−0.04
lfl	−96.06	−53.47	135.13	−0.34	−0.19	0.48
lhm	24.43	37.08	−15.15	0.18	0.27	−0.11
lmb	59.94	−35.83	−94.04	1.60	−0.95	−2.51
lsv	−74.81	−121.66	−95.35	−0.47	−0.76	−0.60
ltv	195.85	−113.24	−195.16	0.80	−0.46	−0.80
lwg	−18.43	27.79	5.98	−1.28	1.93	0.42
mta	115.19	−17.23	159.81	0.83	−0.12	1.16
mtb	72.00	72.21	118.62	0.49	0.49	0.81
mtc	−40.75	−144.59	−73.53	−0.16	−0.57	−0.29
ovp	−245.01	−54.42	150.43	−5.85	−1.30	3.59
spa	−59.46	138.88	151.21	−0.20	0.47	0.51
svf	134.46	76.00	−137.42	2.82	1.59	−2.88
wsv	157.47	29.18	262.31	0.18	0.03	0.31
wwg	17.25	25.26	6.42	0.75	1.09	0.28

with the other variates. Interpretation of this variate is not clear, as a number of different characters from different body regions contributed strongly to this axis. *Trichogramma minutum* and *T. californicum* were weakly separated from *T. platneri* and *T. exiguum* along the third canonical variate (Fig. 5B). It is similar to the first variate in that ovipositor length contributed very strongly, with high loadings involving metatibial length, 1st metatarsal segment length (mta), and 2nd valvifer length. Even though this variate and the first appear to represent similar sources of variation, it is certain that they do not because canonical variate analysis requires that each variate be uncorrelated with the others.

Resampling: Resampling to determine appropriate rates for error of identification was done for cultures of *T. minutum* and *T. platneri* alone, because the method requires a larger sample size of populations relative to specimens than was practical for *T. californicum* and *T. exiguum*, with the result that resampling error rates would tend to be misleadingly high for those species. There was an overall resampling error rate of 11.76% for males and 29.44% for females (Table 12). Specimen misclassification is more common for certain cultures than for others, and was much higher in females than in males. Ambiguous results (40% error rate or more) for both sexes were rare, occurring in only 2 of the 37 cultures analyzed (MCVA, MWTV), which were also the only cultures ambiguously classified using males. Males of most of the cultures (24) were identified with no errors. A total of 11 cultures were ambiguously classified or misclassified in females, with highly misleading results in some cases. Sample size was 3 or less in 4 of the 11 cultures, but 8 out of 10 females were misidentified from PPND, while none of the 5 males was misidentified. This is taken as a strong indication that, despite the possibility of classifying females using these results, males should be used for more accurate determination.

Discriminant analysis test class: Males and females of the five cultures of *T. californicum* without reproductive data were analyzed as separate test classes classified using results from the analyses of the calibration datasets. Among these were the ho-

Table 12. Error rates of resampling cultures in the canonical variate analyses of males and females compared with the error rates from the unmodified analyses. Sample sizes are those in Table 1.

	# Misclassified in calibration		# Misclassified in resampling		Resampling % error	
Locality	Male	Female	Male	Female	Male	Female
T. minutum	4	21	13	38	12.75	31.15
MABQ	0	1	0	1	0	20
MBGS	0	2	0	2	0	25
MBFY	1	0	1	0	33.3	0
MCVA	0	0	5	6	50	60
MCLB	0	0	0	0	0	0
MDRY	0	1	0	2	0	28.57
MFFD	0	1	0	1	0	16.67
MFRU	0	4	1	5	25	83.33
MHND	0	1	0	2	0	28.57
MKNB	0	1	0	2	0	22.22
MKKW	—	1	—	1	—	25
MMDS	0	0	0	1	0	16.67
MEAD	0	3	0	4	0	66.67
MONT	0	1	0	2	0	100
MRHH	0	0	0	0	0	0
MSPL	0	0	0	2	0	25
MSMT	0	1	0	1	0	20
MSMN	0	2	0	2	0	66.67
MSGD	0	0	1	1	25	50
MTRP	0	0	0	2	0	25
MWEN	0	1	1	1	20	25
MWTV	3	1	4	1	40	50
T. platneri	1	13	7	25	10.29	27.17
PBCK	0	0	0	2	0	33.33
PCLV	0	0	1	0	20	0
PCHL	0	0	1	0	20	0
PELT	0	1	0	1	0	25
PGRB	0	1	0	2	0	66.67
PGRN	0	0	0	0	0	0
PJRV	0	0	0	0	0	0
PJUL	0	2	0	3	0	75
PLIB	0	3	1	4	20	44.44
PNWC	1	0	0	0	0	0
PPND	0	5	0	8	0	80
PRV1	0	0	2	0	22.22	0
PSUM	0	0	1	0	25	0
PWWL	0	0	0	2	0	33.33
PWTS	0	1	1	3	20	30
Total all cultures	5	34	20	63	11.76	29.44

lotype male and allotype female from Alturas, CA (CAAL). Of 12 males and 6 females (Table 1), the only misidentified male was one from CAGB, which was identified as *T. platneri*. The holotype male was identified as *T. californicum* with 100% certainty, but the allotype female was identified as *T. minutum*. The only other misidentified female was a specimen from CAGB, which was identified as *T. exiguum*.

DISCUSSION

In males, only species of the *T. minutum* complex could not be distinguished with

high confidence using univariate or bivariate analyses, but in all cases where such separation was possible the differences between species were very slight, being measured in thousandths of a millimeter. We do not recommend using these results by themselves for diagnosis, as the already established morphological characters (Pinto 1999) are no less accurate and are in most cases as easy to assess. The partially discriminating ratios given for *T. minutum* complex males and for females of all four species should prove more useful, but the probability of error even in the best of these cases is so high that final diagnosis should not be performed using these characters alone.

The results of the principal component analyses were at best only slightly more diagnostic than the best univariate and bivariate separations. This is not surprising considering that most diagnostic components show strong loadings of the variables singled out as diagnostic in univariate and bivariate analyses. The lack of complete separation between *T. californicum* and *T. platneri* indicates that morphological overlap between the two species is a reality, at least where large specimens of *T. californicum* and small specimens of *T. platneri* are concerned.

Neither males nor females of *T. minutum* and *T. platneri* could be separated using the principal component results, and canonical variate analysis separated them only with some overlap and with a higher degree of accuracy for males over females. These morphological data alone do not clearly support the notion of these as distinct species, but the species are clearly segregated by allozymic data and mutual reproductive incompatibility (Pinto et al. 1992, Burks and Pinto 2002). These data provide for the first time a morphological means of identifying *T. minutum* and *T. platneri*, albeit with some error, and they should facilitate quality control in insectaries that rear both species and in biological control programs that potentially involve both species. Identification remains difficult, however, requiring nineteen measurements for each male specimen, sixteen for each female specimen, and canonical variate analysis. The measurements must be accurate to a thousandth of a millimeter, and all relevant features must be clearly discernable and not distorted for each specimen. It is also recommended that ten specimens, preferably males, of each culture or population be measured to avoid misclassification. These difficulties make morphological identification about as difficult as identification using electrophoresis or crossing with cultures of known identity, and with less accuracy. Nevertheless, situations exist in which morphological identification is necessary, such as when dealing with dead specimens that are not preserved properly for electrophoresis.

One of the major questions in the taxonomy of *Trichogramma* important to biological control is whether the difference between *T. minutum* and *T. platneri* is one of species rank. These results do not answer that question, but they provide more information. Previously, the difference between the two was electrophoretic differences at two allozymic loci and mutual reproductive incompatibility (Pinto et al. 1991, 1992). Stouthamer et al. (2000) recently found that the two species did not differ in ITS2 sequence, which is a genetic region capable of separating morphologically distinct species of *Trichogramma* (Stouthamer pers. comm.). Canonical variate analysis indicates that there are morphological differences, but these differences are complex and involve broad overlap, and we do not consider separations found in canonical variate analysis alone as strong evidence for species separation because of the strong bias for class separation inherent in the method (Lance et al. 2000). This does not necessarily imply that *T. minutum* and *T. platneri* are not distinct species, but that the morphological difference between them is insufficient to sug-

gest that they are fully discrete entities. However, these results can be used to identify *Trichogramma* cultures and field-collected populations. The culture-level resampling results (Table 12) give an overall error rate that should approximate the error encountered in classifying new specimens using the results of this study. Specimens suspected of belonging to species not included in this study can be singled out by treating them as separate groups in the species class variable, but corroborating evidence is ultimately necessary to confirm their identity and distinctness.

KEY TO MALE *TRICHOGRAMMA* ANALYZED IN THIS STUDY

[This identification key can be used as a supplement to the key to North American males of *Trichogramma* (Pinto 1999). The canonical variates must be calculated using the product of the raw coefficients and measurements, and corrected for the additive constant generated from the analyses of the males calibration dataset (Table 9). Identification is also possible using discriminant analysis, which is preferable for identifying females and *T. minutum* complex males. Percentages given in parentheses for certain values indicate the proportion of applicable specimens in the calibration dataset for which the statement holds true.]

1. Longest flagelliform antennal setal length (lfs; 21–23, Fig. 1) ≤ 0.7 mm (94%). CAN1 < −3.1 (98%). If lfs > 0.7 mm then CAN1 < −4 2
1′. Longest flagelliform antennal setal length > 0.7 mm (99%). CAN1 > −2.65 (100%) 3
2. Ratio of stigmal vein length (53–54, Fig. 1) to apical distance in genital capsule (10–12, Fig. 1) (lsv/apd) ≤ 1.4 (100%). CAN2 < −1.3 (100%) ..... ***T. californicum***
2′. Ratio of stigmal vein length to apical distance > 1.4 (95%). CAN2 > −0.25 (100%) ..... ***T. exiguum***
3. Ratio of longest flagelliform seta length (lfs; 21–23, Fig. 1) to fore wing width (59–60, Fig. 1) (lfs/wwg) > 0.28 (79%). CAN3 > −0.35 (82%) ..... ***T. minutum***
3′. Ratio of longest flagelliform seta length to fore wing width (lfs/wwg) ≤ 0.28 (78%). CAN3 < −0.34 (88%) ..... ***T. platneri***

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